NIH Phase 1: Point Source Ozonation to Minimize Antibiotic Resistance

Sponsor: BlueInGreen, LLC

Project Period: September 30, 2005 – September 29, 2006

Final Report

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Summary

This project was to provide analytical determination for the concentrations of residual fluoroquinolone antibiotics after ozonation treatment using the published method by the PI (Renew and Huang, 2004, *J. Chromatogr. A.*, 1042, 113-121). The overall objective of the project was to assess whether micro-bubble ozonation was an effective mean t o remove emerging contaminants such as fluoroquinolone antibiotics in water. The analyses were conducted for five different sets of batch tests. The analytical results of these samples were reported previously to the sponsor and are summarized in this report. In general, high percentage removal was seen by this ozonation approach.

A. Analytical Results of 07/14/06:

Sample descriptions:

Sample#	Sample description
Sample 1	Dechlorinated tap, 988 mL
Sample 2	1:10 waste sample with no FQ's, 1.0 L - Add 5.844g NaCl to 1L - Add phosphoric acid to ~2.5pH - Filter through 0.7µm glass fiber filter
Sample 3	Dechlorinated tap water with FQ's spike, low concentration, send 1.0 L - Add 11.688g NaCl to 2L - Add phosphoric acid to ~2.5pH - Add 20µL of Levo. Stock (1000mg/L) - Add 80µL of Cipro. Stock (250mg/L)
Sample 4	Waste sample high concentration, 40mL amber vial (Chemical addition and FQ addition based on 1 L) - Add 5.844g NaCl to 1L - Add phosphoric acid to ~2.5pH - Add 10mL of Levo. Stock (1000mg/L) - Add 40mL of Cipro. Stock (250mg/L) - Filter through 0.7µm glass fiber filter

- Samples 1-3 were concentrated by solid phase extraction (SPE) prior to analysese.
- Sample 4 was analyzed directly without SPE or any pretreatment.

SPE: Each sample (988-1000 mL) was extracted through a 500 mg anion-exchange cartridge stacked on top of a 500 mg hydrophilic-lipophilic balance cartridge. Each sample was amended with 10 µg/L lomefloxacin prior to extraction for evaluation of extraction recoveries. After the extraction, 1 mg/L norfloxacin was spiked into the concentrated samples as an internal standard to account for signal suppression effects in different water matrices.

Results

I. Samples concentrated by SPE:

	Norfloxacin	Ciprofloxacin			Levofloxacin			Lomefloxacin		
Sample #	$Area_{Istd}$	Area	$Area_i / Area_{Istd}$	Conc.(mg/L)	Area	$Area_i / Area_{Istd}$	Conc.(mg/L)	Area	$Area_i / Area_{Istd}$	Conc.(mg/L)
Sample 1	5167447.5	ND	ND	**ND**	ND	ND	**ND**	69189216	13.39	**8.10**
Sample 2	2956971	ND	ND	**ND**	ND	ND	**ND**	9378519	3.17	**2.10**
Sample 3	6096668.5	6748697	1.11	**1.18**	44758832	7.34	**4.56**	52934660	8.68	**5.33**

ND = not detectable.

Recovery calculation

Sample #	Concentrated factor (CF)	Lomefloxacin (µg/L) corrected by CF	Recovery %	Concentration corrected by recovery	
				Ciprofloxacin (µg/L)	Levofloxacin (µg/L)
Sample 1	988	8.00	80.04	**ND**	**ND**
Sample 2	1000	2.10	20.95	**ND**	**ND**
Sample 3	1000	5.33	53.34	**2.22**	**8.55**

II. Samples without SPE:

Sample#	Ciprofloxacin		Ofloxacin	
	Area	Conc.(mg/L)	Area	Conc.(mg/L)
Sample 4	29540820	**2.78**	54620428	**3.49**

Summary:

1. Ciprofloxacin and levofloxacin were not detected in the unspiked dechlorinated tap water or wastewater.
2. Ciprofloxacin and levofloxacin were determined to be 2.78 mg/L and 3.49 mg/L in the spiked dechlorinated tap water. These concentrations were lower than the indicated ~10 mg/L spiking concentration. Degradation of the FQs is unlikely the cause. This may be due to errors in the stocks.

3. The low recovery of Sample 2 is likely due to human errors in the SPE procedures (i.e,, during the the blow-down step). The recovery is expected to improve the next time.
4. Overall, accurate analyses of the FQs for study can be conducted in the selected matrices.

Appendix:

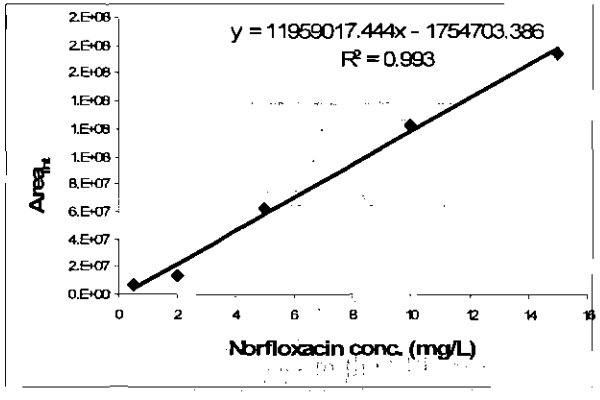
Calibration curve:

Standards of fluoroquinolones

		Peak Area			
		Norfloxacin	Ciprofloxacin	Ofloxacin	Lomefloxacin
	mg/L	MW320	MW332	MW362	MW352
S1	0.5	7348863	7532340	8927217	10020725
S2	2	13599119	14087239	20648978	21137282
S3	5	62212280	56669268	88743072	84674992
S4	10	122698864	115556520	161615568	158451744
S5	15	174035424	171717120	232969536	246389712

Note: Ofloxacin was used as the standard for levofloxacin in this around of analyses.

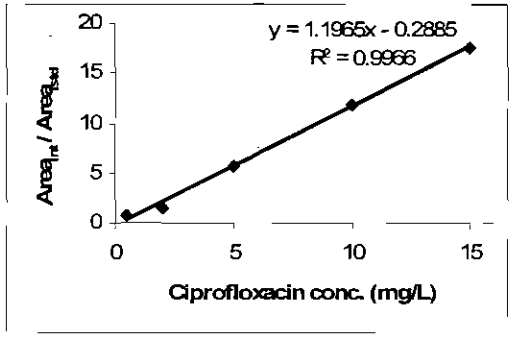
Calibration curve of norfloxacin:

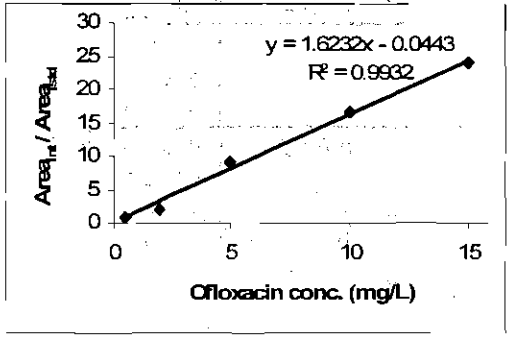


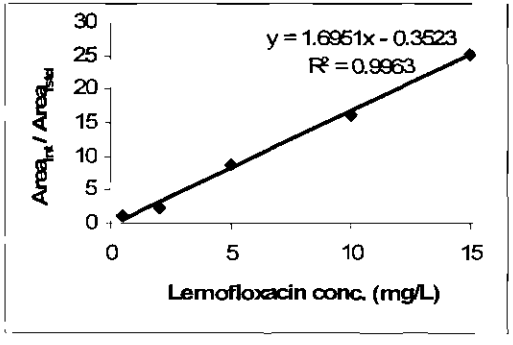
Integrated area of 1 mg/L norfloxacin = 9763424

Norfloxacin were added in all of the samples and standards as an internal standard. The peak area of each FQ (except for norfloxacin) was normalized by the peak area of 1 mg/L norfloxacin (i.e., $Area/Area_{Istd}$). Calibration curves were generated by plotting $Area/Area_{Istd}$ ratios versus the concentration of each FQ.

		$Area/Area_{Istd}$		
		Ciprofloxacin	Ofloxacin	Lemofloxacin
	mg/L	MW332	MW362	MW352
S1	0.5	0.77	0.91	1.03
S2	2	1.44	2.11	2.16
S3	5	5.80	9.09	8.67
S4	10	11.84	16.55	16.23
S5	15	17.59	23.86	25.24







B. Analytical Results of 08/10/06:

Group I

Received Date	Description	pH	Precipitation	Ciprofloxacin		Levofloxacin	
				Integrated Area	Conc. (mg/L)	Integrated Area	Conc. (mg/L)
8.3.06	1:20 Hydoz ~10 mg/L FQ 7.31.06 Initial Rep1 - a	2.41		84865392	4.69	153210272	10.13
8.3.06	1:20 Hydoz ~10 mg/L FQ 7.31.06 Initial Rep1 - b	2.43		84520536	4.66	112742984	6.08
8.3.06	1:20 Hydoz ~10 mg/L FQ 7.31.06 Initial Rep2 - a	2.29		100381488	5.93	153383696	10.15
8.3.06	1:20 Hydoz ~10 mg/L FQ 7.31.06 Initial Rep2 - b	2.34		83172208	4.56	145149968	9.33
8.3.06	1:20 Hydoz ~10 mg/L FQ 7.31.06 Final Rep1 - a	2.84	Yes	85976864	4.78	149775840	9.79
8.3.06	1:20 Hydoz ~10 mg/L FQ 7.31.06 Final Rep1 - b	2.8	Yes	87958008	4.94	118822608	6.69
8.3.06	1:20 Hydoz ~10 mg/L FQ 7.31.06 Final Rep2 - a	2.81	Yes	89059896	5.03	109992968	5.81
8.3.06	1:20 Hydoz ~10 mg/L FQ 7.31.06 Final Rep2 - b	2.8	Yes	78920984	4.22	103408720	5.15
8.3.06	1:10 Hydoz ~10 mg/L FQ 7.31.06 Initial Rep1 - a	2.34		106275880	6.40	126509640	7.46
8.3.06	1:10 Hydoz ~10 mg/L FQ 7.31.06 Initial Rep1 - b	2.34		100570184	5.95	123606136	7.17
8.3.06	1:10 Hydoz ~10 mg/L FQ 7.31.06 Initial Rep2 - a	2.33		109030632	6.62	155565776	10.37
8.3.06	1:10 Hydoz ~10 mg/L FQ 7.31.06 Initial Rep2 - b	2.32		102007640	6.06	118075672	6.62
8.3.06	1:10 Hydoz ~10 mg/L FQ 7.31.06 Final Rep1 - a	2.31	Yes	93755088	5.40	133847912	8.19
8.3.06	1:10 Hydoz ~10 mg/L FQ 7.31.06 Final Rep1 - b	2.58	Yes	77240616	4.08	97381240	4.55
8.3.06	1:10 Hydoz ~10 mg/L FQ 7.31.06 Final Rep2 - a	2.91	Yes	93164224	5.36	105665128	5.38
8.3.06	1:10 Hydoz ~10 mg/L FQ 7.31.06 Final Rep2 - b	2.9	Yes	85817584	4.77	90735200	3.88
8.3.06	1:5 Hydoz ~10 mg/L FQ 8.1.06 Initial Rep1 - a	2.26		85112000	4.71	98188032	4.63
8.3.06	1:5 Hydoz ~10 mg/L FQ 8.1.06 Initial Rep1 - b	2.29		84585024	4.67	96329064	4.44
8.3.06	1:5 Hydoz ~10 mg/L FQ 8.1.06 Initial Rep2 - a	2.25		95653472	5.55	99163200	4.73
8.3.06	1:5 Hydoz ~10 mg/L FQ 8.1.06 Initial Rep2 - b	2.26		82698736	4.52	92514176	4.06
8.3.06	1:5 Hydoz ~10 mg/L FQ 8.1.06 Final Rep1 - a	2.03	Yes	193128560	13.35	47316196	0.87
8.3.06	1:5 Hydoz ~10 mg/L FQ 8.1.06 Final Rep1 - b	1.99	Yes	175536160	11.94	40872156	0.79
8.3.06	1:5 Hydoz ~10 mg/L FQ 8.1.06 Final Rep2 - a	2.12	Yes	64189604	3.04	67393872	1.55
8.3.06	1:5 Hydoz ~10 mg/L FQ 8.1.06 Final Rep2 - b	2.16	Yes	64049896	3.03	65482848	1.36

8.3.06	1:3 Hydoz ~10 mg/L FQ 8.1.06 Initial Rep1 - a	2.3		93362632	5.37	98097496	4.62
8.3.06	1:3 Hydoz ~10 mg/L FQ 8.1.06 Initial Rep1 - b	2.3		91602296	5.23	94864656	4.30
8.3.06	1:3 Hydoz ~10 mg/L FQ 8.1.06 Initial Rep2 - a	2.25	Yes	41085124	1.19	49444064	0.89
8.3.06	1:3 Hydoz ~10 mg/L FQ 8.1.06 Initial Rep2 - b	2.27		75927088	3.98	86183992	3.43
8.3.06	1:3 Hydoz ~10 mg/L FQ 8.1.06 Final Rep2 - a	2.29		86186032	4.80	87028848	3.51
8.3.06	1:3 Hydoz ~10 mg/L FQ 8.1.06 Final Rep2 - b	2.27	Yes	48361296	1.77	50910632	0.91
8.3.06	1:3 Hydoz ~10 mg/L FQ 8.1.06 Final Rep2 - a	2.09	Yes	29021948	0.22	36599096	0.73
8.3.06	1:3 Hydoz ~10 mg/L FQ 8.1.06 Final Rep2 - b	2.12	Yes	39292308	1.05	40720036	0.79

Group II

Received Date	Description	pH	Precipitation	Ciprofloxacin		Levofloxacin	
				Integrated Area	Conc. (mg/L)	Integrated Area	Conc. (mg/L)
8.3.06	Dechlorinated Tap FQ spiked 7.31.06 - a	2.34		95973632	5.58	153798992	10.19
8.3.06	Dechlorinated Tap FQ spiked 7.31.06 - b	2.31		91350264	5.21	113752824	6.19
8.3.06	Dechlorinated Tap 8.1.06 ~10 mg/L FQ Pre Test Sample 8.1.06 2 - a	2.2		75907152	3.97	95029256	4.31
8.3.06	Dechlorinated Tap 8.1.06 ~10 mg/L FQ Pre Test Sample 8.1.06 2 - b	2.22		89611824	5.07	100180176	4.83
8.5.06	Dechlorinated Tap FQ spike ~10 mg/L FQ Pre Test Sample 8.2.06 - a	7.41		68785176	3.40	79709984	2.78
8.5.06	Dechlorinated Tap FQ spike ~10 mg/L FQ Pre Test Sample 8.2.06 - b	2.7	Yes	51408536	2.01	62494840	1.06

Group III

Received Date	Description	pH	Precipitation	Ciprofloxacin		Levofloxacin	
				Integrated Area	Conc. (mg/L)	Integrated Area	Conc. (mg/L)
8.4.06	Bubble Ozone Test Initial Rep1 8.2.06 6.5cfh ozone - a	2.22		61375376	2.81	78696456	2.68
8.4.06	Bubble Ozone Test Initial Rep1 8.2.06 6.5cfh ozone - b	2.26		71045280	3.59	87021192	3.51
8.4.06	Bubble Ozone Test Initial Rep2 8.2.06 6.5cfh ozone - a	2.2		60020184	2.70	77530600	2.56
8.4.06	Bubble Ozone Test Initial Rep2 8.2.06 6.5cfh ozone - b	2.24		62310588	2.89	80140784	2.82
8.5.06	Bubble Ozone Test Final Rep1 8.2.06 6.5cfh ozone - a	2.46	Yes	45928844	1.58	57169260	0.99
8.5.06	Bubble Ozone Test Final Rep1 8.2.06 6.5cfh ozone - b	2.46		43767960	1.40	55756028	0.97

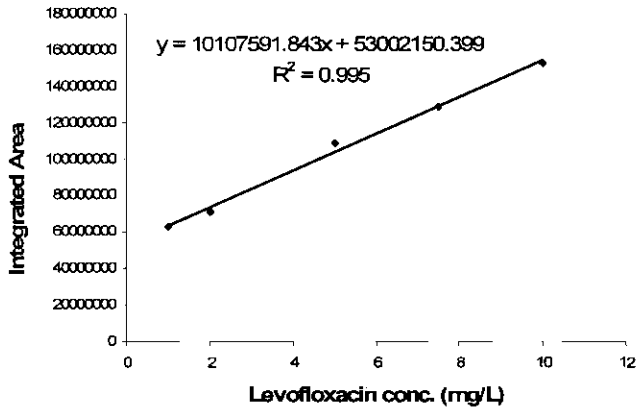
8.5.06	Bubble Ozone Test Final Rep2 8.2.06 6.5cfh ozone - a	2.45	Yes	51355556	2.01	61495192	0.96
8.5.06	Bubble Ozone Test Final Rep2 8.2.06 6.5cfh ozone - b	2.44	Yes	51491596	2.02	62363720	1.05
8.4.06	Bubble Ozone Test Initial Rep1 8.2.06 3.0cfh ozone - a	2.19		57885828	2.53	71303544	1.94
8.4.06	Bubble Ozone Test Initial Rep1 8.2.06 3.0cfh ozone - b	2.24		68132040	3.35	70541416	1.86
8.4.06	Bubble Ozone Test Initial Rep2 8.2.06 3.0cfh ozone - a	2.26		75342608	3.93	82663840	3.08
8.4.06	Bubble Ozone Test Initial Rep2 8.2.06 3.0cfh ozone - b	2.26		68236688	3.36	72576304	2.07
8.5.06	Bubble Ozone Test Final Rep1 8.2.06 3.0cfh ozone - a	2.89	Yes	49695004	1.88	64441900	1.25
8.5.06	Bubble Ozone Test Final Rep1 8.2.06 3.0cfh ozone - b	2.9	Yes	50402432	1.93	62047584	1.01
8.5.06	Bubble Ozone Test Final Rep2 8.2.06 3.0cfh ozone - a	7.35		63288984	2.97	76705944	2.48
8.5.06	Bubble Ozone Test Final Rep2 8.2.06 3.0cfh ozone - b	2.67	Yes	53595672	2.19	63250476	1.14

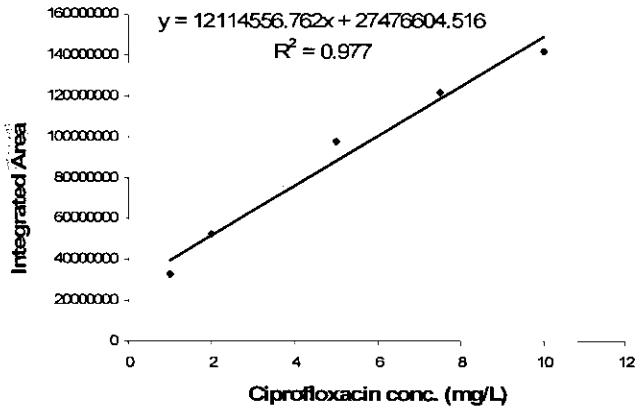
Group IV

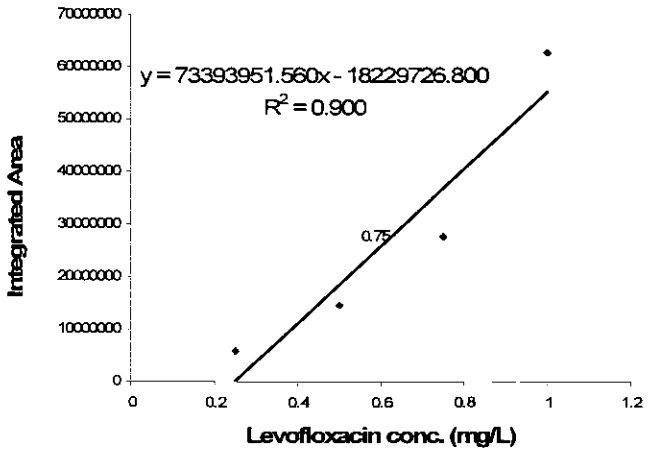
Received Date	Description	pH	Precipitation	Ciprofloxacin		Levofloxacin	
				Integrated Area	Conc. (mg/L)	Integrated Area	Conc. (mg/L)
8.4.06	20 mg/L Chlorine Test 8.2.06 Initial Rep1 - a	2.3		77264864	4.08	85330640	3.34
8.4.06	20 mg/L Chlorine Test 8.2.06 Initial Rep1 - b	2.31		78078832	4.15	85752368	3.39
8.4.06	20 mg/L Chlorine Test 8.2.06 Initial Rep2 - a	2.21		76969960	4.06	82566312	3.07
8.4.06	20 mg/L Chlorine Test 8.2.06 Initial Rep2 - b	2.28		71350690	3.61	78408048	2.65
8.5.06	20 mg/L Chlorine Test 8.2.06 Final Rep1 - a	3.1	Yes	35209896	0.72	66040988	1.41
8.5.06	20 mg/L Chlorine Test 8.2.06 Final Rep1 - b	3.09	Yes	34245888	0.64	64378484	1.25
8.5.06	20 mg/L Chlorine Test 8.2.06 Final Rep2 - a	3.06	Yes	33014624	0.54	64009960	1.21
8.5.06	20 mg/L Chlorine Test 8.2.06 Final Rep2 - b	3.07	Yes	28625118	0.19	60676360	1.03
8.4.06	10 mg/L Chlorine Test 8.2.06 Initial Rep1 - a	2.29		78430912	4.18	83564104	3.17
8.4.06	10 mg/L Chlorine Test 8.2.06 Initial Rep1 - b	2.33		69402808	3.45	78487944	2.66
8.4.06	10 mg/L Chlorine Test 8.2.06 Initial Rep2 - a	2.27		69859216	3.49	75977144	2.41
8.4.06	10 mg/L Chlorine Test 8.2.06 Initial Rep2 - b	2.3		65968748	3.18	73007872	2.11
8.5.06	10 mg/L Chlorine Test 8.2.06 Final Rep1 - a	2.59	Yes	38275656	0.96	67349640	1.55
8.5.06	10 mg/L Chlorine Test 8.2.06 Final Rep1 - b	2.6	Yes	37957824	0.94	63639328	1.17
8.5.06	10 mg/L Chlorine Test 8.2.06 Final Rep2 - a	3.16	Yes	41090572	1.19	58006248	1.00
8.5.06	10 mg/L Chlorine Test 8.2.06 Final Rep2 - b	3.18	Yes	41934944	1.26	69246584	1.73

Calibration standards:

Sample#	mg/L	Integrated Area	
		Ciprofloxacin	Levofloxacin
		MW332	MW362
S1	0.25	2407772.5	5811350.7
S2	0.5	10512725	14507997
S3	0.75	22354160	27656472
S4	1	32688936	62590152
S5	2	52499508	71005392
S6	5	97477864	108285344
S7	7.5	121786712	128267360
S8	10	141851200	152606096







C. Analytical Results of 09/02/06:

Sample descriptions:

Sample #	Sample description	Date	pH	Precipitation
1	Pre-test Sample FQ addition	8/22/2006	2.32	No
2	1:10 Initial Rep1	8/22/2006	2.39	No
3	1:10 Initial Rep2	8/22/2006	2.25	No
4	1:10 Final Rep1	8/22/2006	2.46	No
5	1:10 Final Rep2	8/22/2006	2.46	No
6	1:20 Initial Rep1	8/22/2006	2.34	No
7	1:20 Initial Rep2	8/22/2006	2.24	No
8	1:20 Final Rep1	8/22/2006	1.9	No
9	1:20 Final Rep2	8/22/2006	2.5	No
10	Pre-test Sample FQ spiked	8/23/2006	2.38	No
11	1:3 HYDOZ Initial Rep1	8/23/2006	2.32	No
12	1:3 HYDOZ Initial Rep2	8/23/2006	2.23	No
13	1:3 HYDOZ Final Rep1	8/23/2006	2.93	**Yes**
14	1:3 HYDOZ Final Rep2	8/23/2006	2.42	**Yes**
15	1:5 HYDOZ Initial Rep1	8/23/2006	3.27	No
16	1:5 HYDOZ Initial Rep2	8/23/2006	2.34	No
17	1:5 HYDOZ Final Rep1	8/23/2006	3.04	**Yes**
18	1:5 HYDOZ Final Rep2	8/23/2006	2.47	**Yes**
19	Bubble O_3 3.0 cfh Initial Rep1	8/26/2006	2.7	No
20	Bubble O_3 3.0 cfh Initial Rep2	8/26/2006	2.69	No
21	Bubble O_3 3.0 cfh Final Rep1	8/26/2006	2.71	No
22	Bubble O_3 3.0 cfh Final Rep2	8/26/2006	2.72	No
23	Bubble O_3 6.5 cfh Initial Rep1	8/26/2006	2.57	No
24	Bubble O_3 6.5 cfh Initial Rep2	8/26/2006	2.85	No

25	Bubble O_3 6.5 cfh Final Rep1	8/26/2006	2.81	No
26	Bubble O_3 6.5 cfh Final Rep2	8/26/2006	2.98	No
27	Pre-test Sample FQ spiked	8/28/2006	2.44	No
28	10 mg/L Cl Dosage Initial Rep1	8/28/2006	3.57	**Yes**
29	10 mg/L Cl Dosage Initial Rep2	8/28/2006	2.5	No
30	10 mg/L Cl Dosage Final Rep1	8/28/2006	3.97	**Yes**
31	10 mg/L Cl Dosage Final Rep2	8/28/2006	2.93	**Yes**
32	20 mg/L Cl Dosage Initial Rep1	8/28/2006	3.38	No
33	20 mg/L Cl Dosage Initial Rep2	8/28/2006	2.51	No
34	20 mg/L Cl Dosage Final Rep1	8/28/2006	4.01	**Yes**
35	20 mg/L Cl Dosage Final Rep2	8/28/2006	3.54	No

Samples with precipitation were filtered by 0.7 μm glass filters before solid phase extraction (SPE). All samples (972-1000 mL) were extracted through a 500 mg anion-exchange cartridge stacked on top of a 500 mg hydrophilic-lipophilic balance cartridge. Sample 1 - 26 were amended with 10 μg/L lomefloxacin and Sample 27 - 35 were amended with 20 μg/L lomefloxacin prior to extraction for evaluation of extraction recoveries. After the extraction, 1 mg/L norfloxacin was spiked into the concentrated samples as an internal standard to account for signal suppression effects in different water matrices.

Results:

	Norfloxacin	**Ciprofloxacin**			**Levofloxacin**			**Lomefloxacin**		
Sample #	$Area_{Istd}$	Area	$Area/Area_{Istd}$	Conc.(mg/L)	Area	$Area/Area_{Istd}$	Conc.(mg/L)	Area	$Area/Area_{Istd}$	Conc.(mg/L)
1	280507.6	154376048	550.3	60.327	151396384	539.7	55.3	29462626	105.0	6.8
2	349530	164703216	471.2	51.535	167537088	479.3	48.8	33475480	95.8	6.1
3	252114.1	155274560	615.9	67.610	151927120	602.6	62.0	28485218	113.0	7.4
4	64803.8	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	45039.4	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	345589.6	190796560	552.1	60.521	193691344	560.5	57.5	34808836	100.7	6.5
7	259546.7	155694000	599.9	65.830	160218736	617.3	63.5	30317806	116.8	7.7
8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

9	44603.1	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	303054.5	152400240	502.9	55.053	165662432	546.6	56.0	33404438	110.2	7.2
11	338882	199816368	589.6	64.692	201458096	594.5	61.1	43755116	129.1	8.7
12	330327	202494208	613.0	67.290	198380864	600.6	61.7	42905088	129.9	8.8
13	307623.6	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	348742.2	195657456	561.0	61.515	197698768	566.9	58.2	40425428	115.9	7.7
16	270688.5	180240096	665.9	73.161	181322288	669.9	69.1	39650540	146.5	10.0
17	130526	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	248606	168680576	678.5	74.567	171723520	690.7	71.3	32936594	132.5	9.0
20	234935.7	133072856	566.4	62.113	136287824	580.1	59.6	27115508	115.4	7.6
21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	27284.6	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	256859.8	140425392	546.7	59.922	138481968	539.1	55.2	28297974	110.2	7.2
24	288948.1	147319744	509.8	55.828	158319840	547.9	56.1	33667012	116.5	7.7
25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
26	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
27	289174	130499824	451.3	49.321	145866448	504.4	51.5	54552844	188.7	13.3
28	281790.5	134075736	475.8	52.044	149907792	532.0	54.4	55234960	196.0	13.9
29	266324.3	134012192	503.2	55.088	141685440	531.8	54.3	51649644	193.9	13.7
30	136520.7	ND	ND	ND	ND	ND	ND	ND	ND	ND
31	50540	ND	ND	ND	ND	ND	ND	ND	ND	ND
32	264404.4	136250400	515.3	56.434	142573024	539.2	55.2	53408628	202.0	14.3
33	258476	136153376	526.8	57.706	139324352	539.0	55.2	50355020	194.8	13.8
34	74637	ND	ND	ND	ND	ND	ND	7013482	94.0	6.0
35	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = not detectable.

Recovery calculation

Sample #	Concentrated factor (CF)	Lomefloxacin (μg/L) corrected by CF	Recovery %	Concentration corrected by recovery	
				Ciprofloxacin (μg/L)	Levofloxacin (μg/L)
1	1000	6.8	68.3	88.3	80.9
2	1000	6.1	61.1	84.3	79.9
3	1000	7.4	74.4	90.8	83.2
4	1000	ND	ND	ND	ND
5	1000	ND	ND	ND	ND
6	1000	6.5	65.0	93.2	88.5
7	1000	7.7	77.4	85.1	82.1
8	1000	ND	ND	ND	ND
9	1000	ND	ND	ND	ND
10	1000	7.2	72.3	76.1	77.5
11	1000	8.7	86.9	74.4	70.3
12	1000	8.8	87.5	76.9	70.5
13	972	ND	ND	ND	ND
14	994	ND	ND	ND	ND
15	1000	7.7	76.7	80.2	75.8
16	1000	10.0	100.4	72.9	68.9
17	1000	ND	ND	ND	ND
18	1000	ND	ND	ND	ND
19	1000	9.0	89.5	83.3	79.7
20	1000	7.6	76.3	81.4	78.0
21	1000	ND	ND	ND	ND
22	1000	ND	ND	ND	ND
23	1000	7.2	72.3	82.9	76.4
24	1000	7.7	77.2	72.3	72.8
25	1000	ND	ND	ND	ND
26	1000	ND	ND	ND	ND

27	1000	13.3	66.5	74.2	77.5
28	1000	13.9	69.3	75.1	78.5
29	1000	13.7	68.5	80.4	79.4
30	1000	ND	ND	ND	ND
31	1000	ND	ND	ND	ND
32	1000	14.3	71.7	78.8	77.1
33	1000	13.8	68.9	83.8	80.1
34	1000	6.0	29.9	ND	ND
35	1000	ND	ND	ND	ND

Summary:

5. Solid phase extraction recoveries ranged from 69-93% with reasonably good consistency.
6. Amended lomefloxacin were not detected in the final samples. The disappearance of spiked lomefloxacin is likely caused by oxidation by residual oxidants due to incompletely quenching of the reaction samples.
7. Ciprofloxacin and levofloxacin were not detected in the final samples. Degradation of ciprofloxacin and levofloxacin by residual oxidants over time may also contribute to the disappearance of ciprofloxacin and levofloxacin in the reaction samples.

Appendix:

Calibration curve:

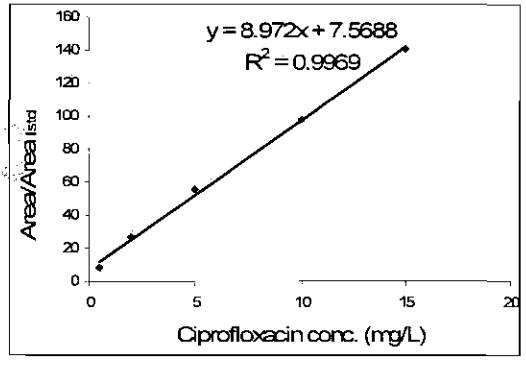
Standards of fluoroquinolones

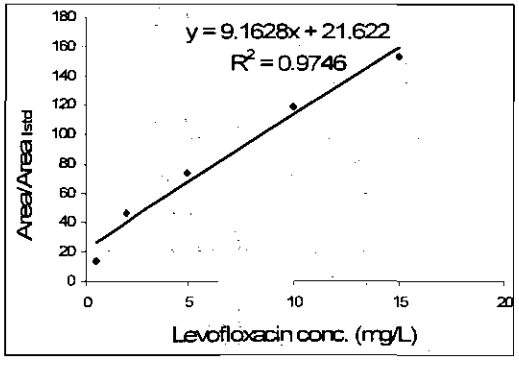
		Peak Area			
		Norfloxacin	Ciprofloxacin	Levofloxacin	Lomefloxacin
	mg/L	MW320	4474806	7678587	7939252
S1	0.5	562489.8	13228203	22991236	23336092
S2	2	490656.6	31076966	41010436	47260768
S3	5	553490.1	53267384	64353464	85861272

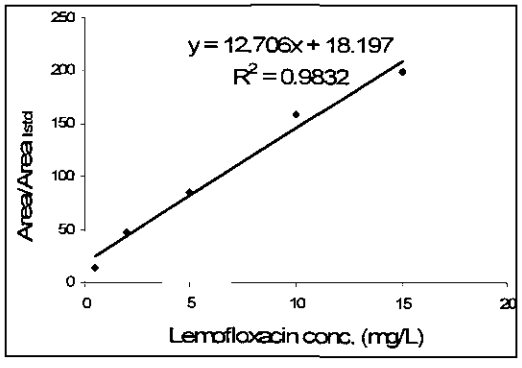
S4	10	543672.1	76145216	82944264	107901472
S5	15	542367.9	171717120	232969536	246389712

Norfloxacin were added in all of the samples and standards as an internal standard. The peak area of each FQ (except for norfloxacin) was normalized by the peak area of 1 mg/L norfloxacin (i.e., Area/$Area_{Istd}$). Calibration curves were generated by plotting Area/$Area_{Istd}$ ratios versus the concentration of each FQ.

		Area/ $Area_{Istd}$		
		Ciprofloxacin	Levofloxacin	Lemofloxacin
	mg/L	MW332	MW362	MW352
S1	0.5	8.0	13.7	14.1
S2	2	27.0	46.9	47.6
S3	5	56.1	74.1	85.4
S4	10	98.0	118.4	157.9
S5	15	140.4	152.9	198.9







D. Analytical Results of 09/26/2006:

Sample descriptions

Sample #	Date	pH	Precipitation
T2	9/12/2006	2.91	No
T3	9/12/2006	3.13	Yes
T4	9/12/2006	2.96	No
T5	9/12/2006	3.01	Yes
T6	9/12/2006	2.78	No
T7	9/12/2006	2.88	Yes
T8	9/12/2006	3.17	No
T9	9/13/2006	2.74	Yes
T10	9/13/2006	2.74	No
T11	9/13/2006	3.24	Yes
T12	9/13/2006	2.82	No
T13	9/13/2006	3.46	Yes
T14	9/13/2006	2.77	No
T15	9/13/2006	3.77	Yes
T16	9/13/2006	2.83	No
T17	9/13/2006	3.14	Yes
T18	9/14/2006	2.74	No
T19	9/14/2006	3.38	Yes
T20	9/14/2006	3.01	No
T21	9/14/2006	3.72	Yes
T22	9/14/2006	3.10	No
T23	9/14/2006	3.65	Yes
T24	9/14/2006	3.18	No
T25	9/14/2006	3.86	Yes

T26	9/19/2006	2.84	No
T27	9/19/2006	3.73	No
T28	9/19/2006	2.88	No
T29	9/19/2006	3.60	Yes
T31	9/19/2006	3.81	Yes
T32	9/19/2006	2.09	No
T33	9/19/2006	3.31	Yes

Samples with precipitation were filtered by 0.7 µm glass filter before solid phase extraction (SPE). All samples (544-1000 mL) were extracted through a 500 mg anion-exchange cartridge stacked on top of a 500 mg hydrophilic-lipophilic balance cartridge. Sample were amended with 15 µg/L lomefloxacin prior to extraction for evaluation of extraction recoveries. After the extraction, 10 mg/L norfloxacin was spiked into the concentrated samples as an internal standard to account for signal suppression effects in different water matrices.

Results:

	Norfloxacin	Ciprofloxacin			Levofloxacin			Lomefloxacin		
Sample #	$Area_{Istd}$	Area	$Area_i / Area_{Istd}$	Conc.(mg/L)	Area	$Area_i / Area_{Istd}$	Conc.(mg/L)	Area	$Area_i / Area_{Istd}$	Conc.(mg/L)
T2	8368320	29156016	3.5	36.13	41560628	5.0	34.35	15413229	1.8	11.88
T3	7277189	2514360	0.3	3.27	17793974	2.4	16.04	6448455	0.9	4.69
T4	8962956	32045050	3.6	37.08	46362536	5.2	35.84	18158254	2.0	13.26
T5	6703785	2625263	0.4	3.75	21705792	3.2	21.79	7599330	1.1	6.55
T6	8372629	31715830	3.8	39.31	52404780	6.3	43.73	17092532	2.0	13.38
T7	7202985	ND	ND	ND	ND	ND	ND	ND	ND	ND
T8	9080636	30224354	3.3	34.50	47331172	5.2	36.13	17671046	1.9	12.66
T9	7601765	ND	ND	ND	5275494	0.7	3.32	14811278	1.9	12.68
T10	6405200	21328858	3.3	34.52	32896362	5.1	35.58	12849258	2.0	13.11
T11	5062917	ND	ND	ND	ND	ND	ND	ND	ND	ND
T12	8690580	22471694	2.6	26.72	36650872	4.2	28.91	14819736	1.7	10.85
T13	4516581	ND	ND	ND	5261409	1.2	6.74	2435620	0.5	2.08

T14	8383128	18637732	2.2	22.93	33319116	4.0	27.14	13739848	1.6	10.35
T15	7352716	2078785	0.3	2.61	ND	ND	ND	9550360	1.3	7.79
T16	7677074	19803384	2.6	26.66	36713988	4.8	33.01	14945212	1.9	12.67
T17	6544585	ND	ND	ND	ND	ND	ND	ND	ND	ND
T18	7763831	30586330	3.9	40.90	44408120	5.7	39.82	15583503	2.0	13.12
T19	5116759	2877766	0.6	5.54	21630068	4.2	28.98	7648693	1.5	9.27
T20	7635052	27200830	3.6	36.95	42018384	5.5	38.25	16064508	2.1	13.85
T21	5665418	12720359	2.2	23.16	30149920	5.3	36.93	11346432	2.0	13.09
T22	9275749	29482910	3.2	32.93	47488248	5.1	35.46	16979312	1.8	11.79
T23	5429067	8765511	1.6	16.55	29072142	5.4	37.17	10306907	1.9	12.30
T24	7493763	26774134	3.6	37.06	42782920	5.7	39.74	15646282	2.1	13.73
T25	6820361	9129087	1.3	13.66	31197760	4.6	31.50	11210018	1.6	10.39
T26	9820726	19131766	1.9	20.05	27697388	2.8	18.76	16932466	1.7	10.99
T27	6076800	8913758	1.5	15.01	27431690	4.5	31.06	10369371	1.7	10.86
T28	7254657	21901992	3.0	31.26	32876942	4.5	31.19	12615877	1.7	11.10
T29	5819139	11778487	2.0	20.84	27747386	4.8	32.91	10351645	1.8	11.40
T31	5178971	14630146	2.8	29.23	30410218	5.9	40.92	10354684	2.0	13.06
T32	6893653	23071740	3.3	34.69	35654020	5.2	35.84	12997677	1.9	12.21
T33	4970432	2003595	0.4	3.87	2094426	0.4	1.34	7246906	1.5	8.99

ND = not detectable.

Recovery calculation

Sample #	Concentrated factor (CF)	Lomefloxacin (µg/L) corrected by CF	Recovery %	Concentration corrected by recovery	
				Ciprofloxacin (µg/L)	Levofloxacin (µg/L)
T2	1000	11.9	79.2	45.6	43.4
T3	1000	4.7	31.2	10.5	51.3
T4	1000	13.3	88.4	41.9	40.5
T5	1000	6.5	43.7	8.6	49.9
T6	1000	13.4	89.2	44.1	49.0
T7	1000	ND	ND	ND	ND
T8	1000	12.7	84.4	40.9	42.8

T9	1000	12.7	84.5	ND	3.9
T10	1000	13.1	87.4	39.5	40.7
T11	1000	ND	ND	ND	ND
T12	1000	10.8	72.3	36.9	40.0
T13	1000	2.1	13.8	ND	48.7
T14	1000	10.4	69.0	33.2	39.3
T15	1000	7.8	51.9	5.0	ND
T16	1000	12.7	84.4	31.6	39.1
T17	1000	ND	ND	ND	ND
T18	1000	13.1	87.5	46.8	45.5
T19	1000	9.3	61.8	9.0	46.9
T20	1000	13.8	92.3	40.0	41.4
T21	1000	13.1	87.3	26.5	42.3
T22	1000	11.8	78.6	41.9	45.1
T23	1000	12.3	82.0	20.2	45.3
T24	1000	13.7	91.5	40.5	43.4
T25	1000	10.4	69.2	19.7	45.5
T26	544	20.2	73.3	27.4	25.6
T27	1000	10.9	72.4	20.7	42.9
T28	1000	11.1	74.0	42.2	42.1
T29	1000	11.4	76.0	27.4	43.3
T31	1000	13.1	87.1	33.6	47.0
T32	1000	12.2	81.4	42.6	44.0
T33	1000	9.0	59.9	6.5	2.2

-Amended lomefloxacin were not detected in T7, T11 and T17 samples, and T3, T5 and T13 have low recovery.

Product identification

From the preliminary results, the remaining ciprofloxacin concentration in the T3 sample is low compared to the T2 sample. Thus, T2 and T3 can be an appropriate pair for product identification. Figure 1 shows the LC/MS SIM chromatograms of the T2 sample, T3 sample, and a sample of ciprofloxacin oxidation product generated from reaction with HOCl. A product peak was identified by comparing the T2 and T3 chromatograms (Figure 1(a) and 1(b)). The retention time of this product peak matches well with that of the ciprofloxacin oxidation product by HOCl (Figure 1 (c)). Figure 2 shows the MS spectrum of the ciprofloxacin oxidation product, indicating that it is a m/z 263 compound resulted from dealkylation of the piperazine ring (Figure 3). The match in retention time and m/z ratio confirmed that the product in the T3 sample is a ciprofloxacin analog after full dealkylation of the piperazine ring (Product 1, M.W. = 262, shown in Figure 3). It is hypothesized that reaction of ciprofloxacin with ozone results first in partial dealkylation of the ciprofloxacin's piperazine ring, followed by complete dealkylation of the piperazine ring to yield the final Product 1 (Figure 3).

Remaining levofloxacin concentrations in all of the samples were relatively high (when compared to the remaining ciprofloxacin concentrations). These results indicate that levofloxacin is more difficult to degrade than ciprofloxacin. Due to limited degradation of the parent levofloxacin, there were not enough products formed and thus no detectable products could be identified by LC/MS. However, based on the similar structures of ciprofloxacin and levofloxacin, a similar degradation pathway may occur for levofloxacin. In this case, the hypothesized degradation pathway and product for levofloxacin is shown in Figure 4.

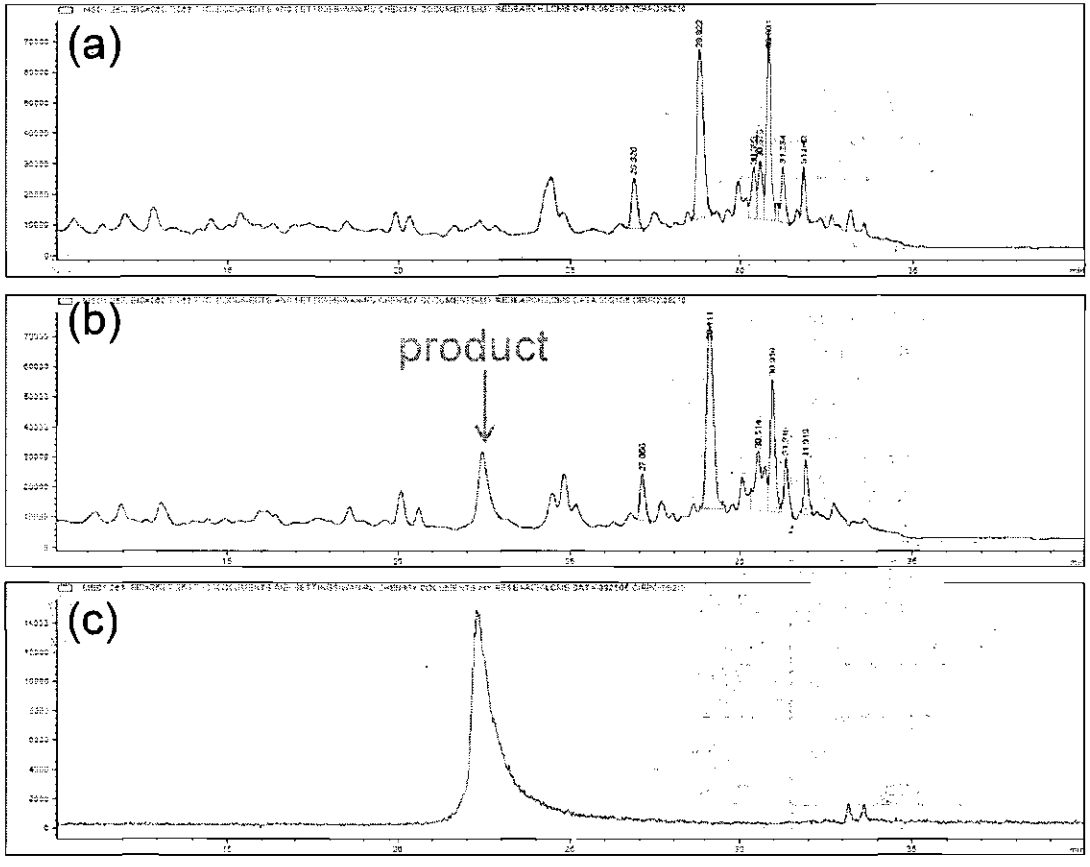


Figure 1 HPLC/MSD chromatograms (SIM: m/z = 263). (a) T2 sample, (b) T3 sample, (c) Oxidation product of ciprofloxacin by HOCl.

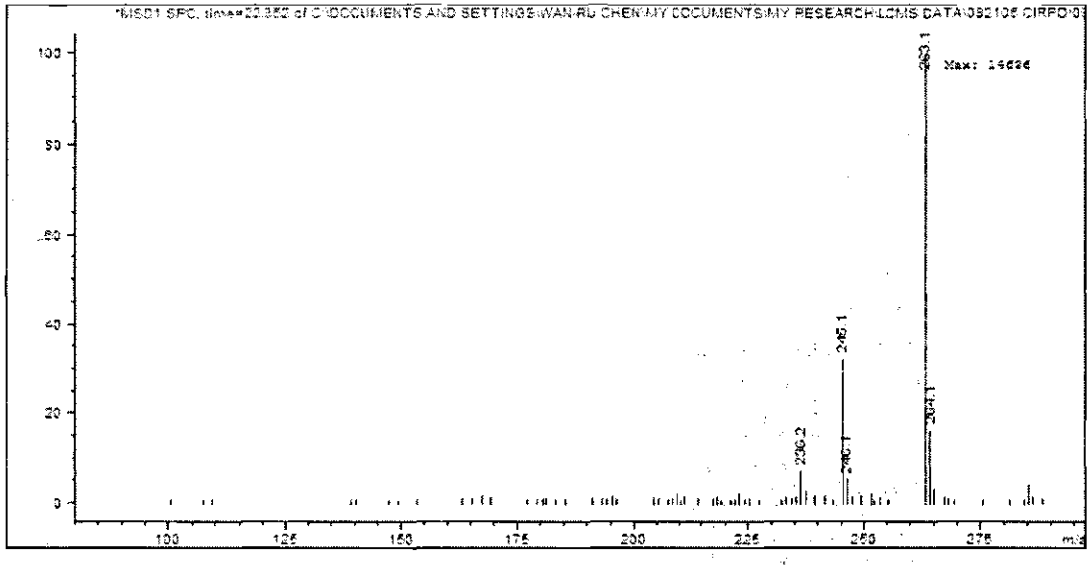


Figure 2 MS spectrum of ciprofloxacin oxidation product.

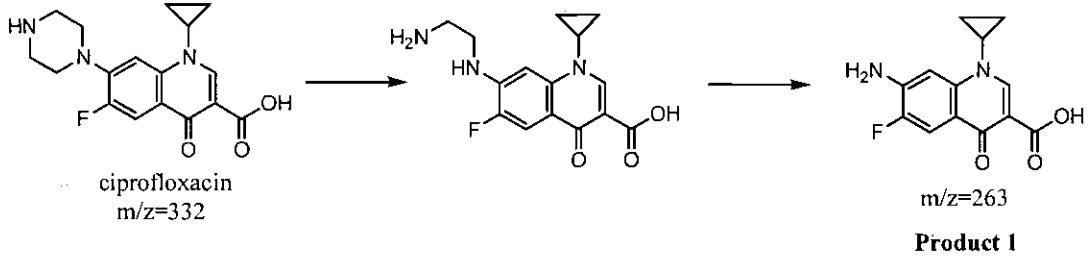


Figure 3 Proposed oxidation products of ciprofloxacin.

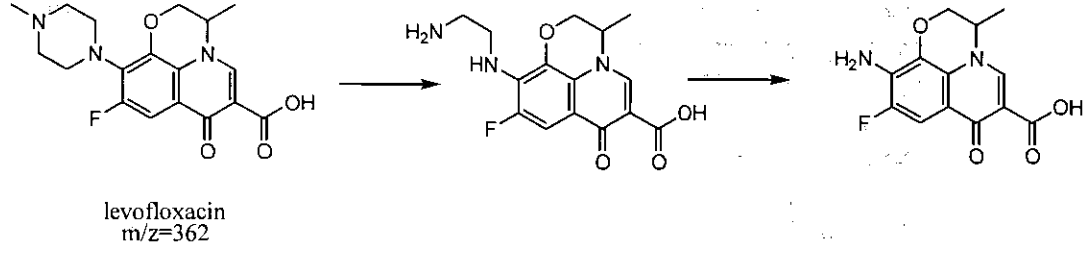


Figure 4 Predicted oxidation products of levofloxacin.

Appendix:

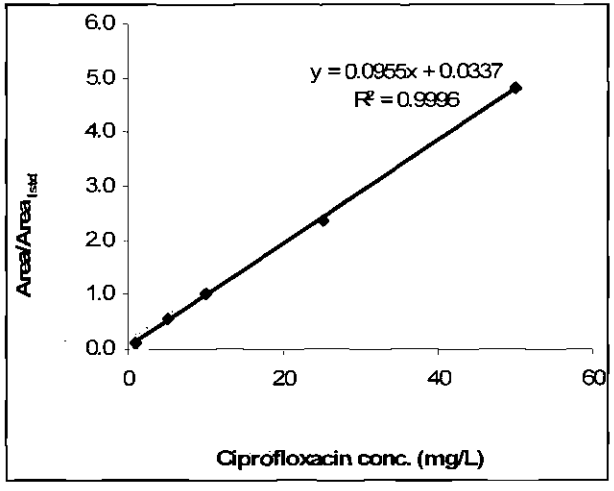
Calibration curve:

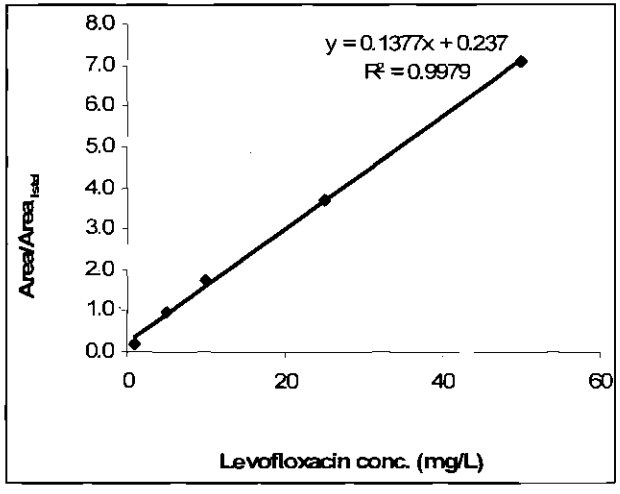
Standards of fluoroquinolones

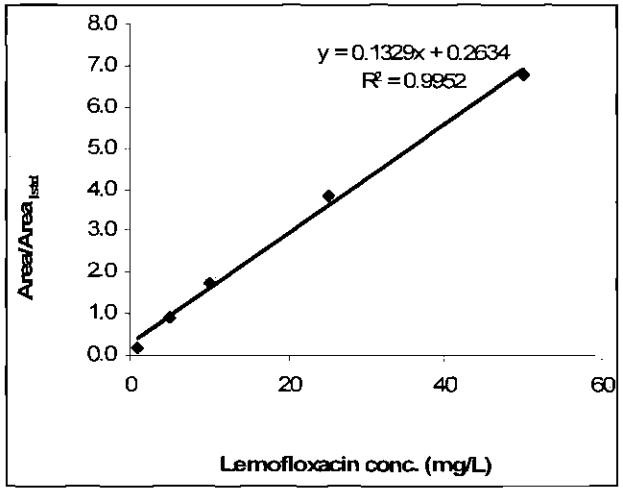
		Peak Area			
		Norfloxacin	Ciprofloxacin	Levofloxacin	Lomefloxacin
	mg/L	MW320	MW332	MW362	MW352
S1	1	18647460	1649085	3443171	3439607
S2	5	16914994	9211777	16782620	15493539
S3	10	16655911	17064226	29281522	28605386
S4	25	17428140	41509176	64526240	66631236
S5	50	17200340	82841104	121776672	116458848

Norfloxacin were added in all of the samples and standards as an internal standard. The peak area of each FQ (except for norfloxacin) was normalized by the peak area of 10 mg/L norfloxacin (i.e., $Area/Area_{Istd}$). Calibration curves were generated by plotting $Area/Area_{Istd}$ ratios versus the concentration of each FQ.

		$Area_i/Area_{Istd}$		
		Ciprofloxacin	Levofloxacin	Lemofloxacin
	mg/L	MW332	MW362	MW352
S1	1	0.09	0.18	0.18
S2	5	0.54	0.99	0.92
S3	10	1.02	1.76	1.72
S4	25	2.38	3.70	3.82
S5	50	4.82	7.08	6.77


y = 0.0955x + 0.0337
R² = 0.9996
Area/Area$_{Istd}$
Ciprofloxacin conc. (mg/L)


y = 0.1377x + 0.237
R² = 0.9979
Area/Area$_{Istd}$
Levofloxacin conc. (mg/L)


y = 0.1329x + 0.2634
R² = 0.9952
Area/Area$_{Istd}$
Lemofloxacin conc. (mg/L)

E. Analytical Results of 10/04/06:

Sample descriptions

Sample #	Date	pH	Precipitation
T34	09/27/06	2.48	No
T35	09/27/06	3.35	Yes
T36	09/27/06	3.20	No
T37	09/27/06	3.33	Yes
T38	09/27/06	2.81	No
T39	09/27/06	3.24	Yes
T40	09/27/06	3.21	No
T41	09/27/06	3.09	Yes

Samples with precipitation were filtered by 0.7 μm glass fiber filters before solid phase extraction (SPE). Each sample was extracted through a 500 mg anion-exchange cartridge stacked on top of a 500 mg hydrophilic-lipophilic balance cartridge. Samples were amended with 15 μg/L lomefloxacin prior to extraction for evaluation of extraction recoveries. After the extraction, 10 mg/L norfloxacin was spiked into the concentrated samples as an internal standard to account for signal suppression effects in different water matrices.

Results:

	Norfloxacin	Ciprofloxacin			Levofloxacin			Lomefloxacin		
Sample #	$Area_{Istd}$	Area	$Area_i / Area_{Istd}$	Conc.(mg/L)	Area	$Area_i / Area_{Istd}$	Conc.(mg/L)	Area	$Area_i / Area_{Istd}$	Conc.(mg/L)
T34	8242603	26412584	3.2	33.20	39069016	4.7	32.70	14461174	1.8	11.22
T35	6276073	ND	ND	ND	11089077	1.8	11.11	3882611	0.6	2.67
T36	9726196	28723612	3.0	30.57	42669028	4.4	30.14	16356123	1.7	10.67
T37	7258963	ND	ND	ND	ND	ND	ND	ND	ND	ND
T38	8959744	28156886	3.1	32.55	43216320	4.8	33.31	16217826	1.8	11.64
T39	6944254	ND	ND	ND	ND	ND	ND	ND	ND	ND
T40	9426068	33391374	3.5	36.74	49353308	5.2	36.30	18345684	1.9	12.66
T41	6531281	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = not detectable.

Recovery calculation

Sample #	Concentrated factor (CF)	Lomefloxacin (µg/L) corrected by CF	Recovery %	Concentration corrected by recovery	
				Ciprofloxacin (µg/L)	Levofloxacin (µg/L)
T34	1000	11.2	74.8	44.4	43.7
T35	1000	2.7	17.8	ND	62.3
T36	1000	10.7	71.1	43.0	42.4
T37	1000	ND	ND	ND	ND
T38	1000	11.6	77.6	42.0	42.9
T39	1000	ND	ND	ND	ND
T40	1000	12.7	84.4	43.5	43.0
T41	1000	ND	ND	ND	ND

-Amended lomefloxacin were not detected in the T37, T39 and T41 samples and the T35 sample has lower recovery.

Appendix:

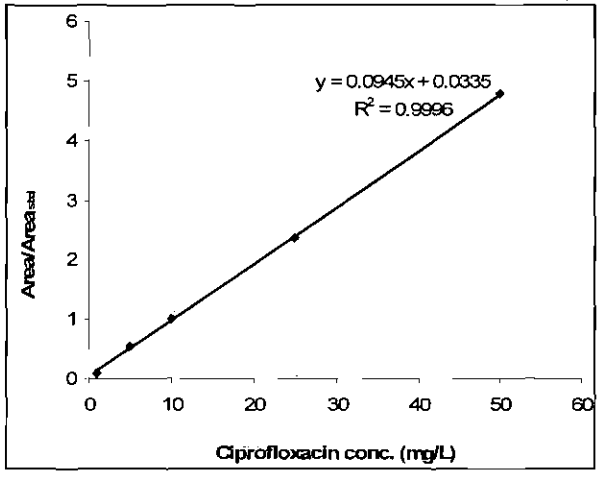
Calibration curve:

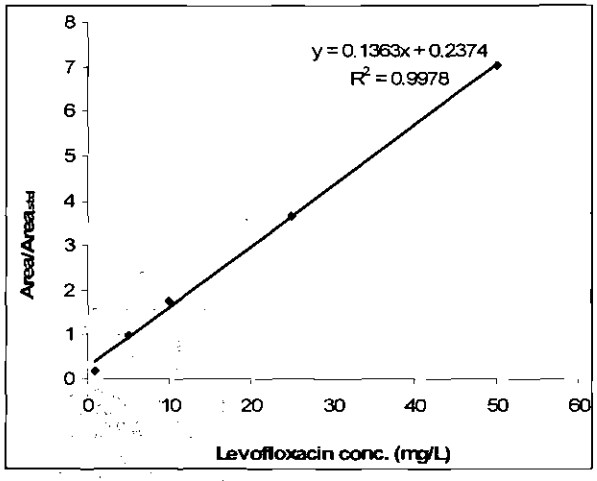
Standards of fluoroquinolones

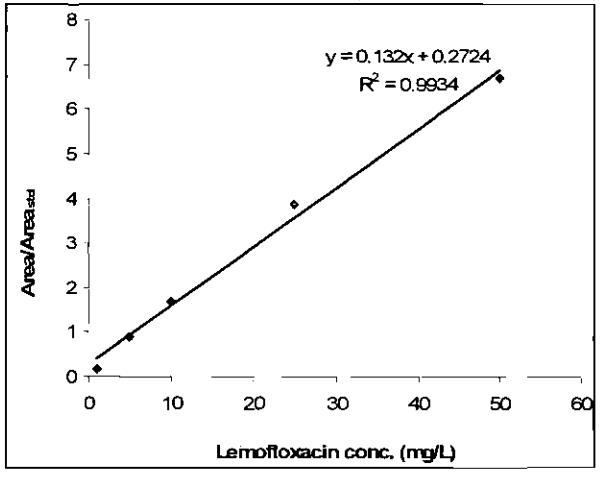
		Peak Area			
		Norfloxacin	Ciprofloxacin	Levofloxacin	Lomefloxacin
	mg/L	MW320	MW332	MW362	MW352
S1	1	18460985	1632594	3408739	3405211
S2	5	16745844	9119659	16614794	15338604
S3	10	16489352	16893584	28988707	28319332
S4	25	17253859	41094084	63880978	65964924
S5	50	17028337	82012693	120558905	115294260

Norfloxacin were added in all of the samples and standards as an internal standard. The peak area of each FQ (except for norfloxacin) was normalized by the peak area of 10 mg/L norfloxacin (i.e., $Area/Area_{Istd}$). Calibration curves were generated by plotting $Area/Area_{Istd}$ ratios versus the concentration of each FQ.

		$Area/Area_{Istd}$		
		Ciprofloxacin	Levofloxacin	Lemofloxacin
	mg/L	MW332	MW362	MW352
S1	1	0.09	0.18	0.18
S2	5	0.54	0.98	0.91
S3	10	1.01	1.74	1.70
S4	25	2.36	3.67	3.78
S5	50	4.77	7.01	6.70


y = 0.0945x + 0.0335
R² = 0.9996
Area/Area std
Ciprofloxacin conc. (mg/L)


y = 0.1363x + 0.2374
R² = 0.9978
Area/Area std
Levofloxacin conc. (mg/L)


y = 0.132x + 0.2724
R² = 0.9934
Area/Area std
Lemofloxacin conc. (mg/L)